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ab270048 His-Tag Protein Expression Check Kit

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes 4003-0030.

View ab270048
His-Tag Protein Expression Check Kit:
www.abcam.com/ab270048
(use www.abcam.cn/ab270048 for China, or http://www.abcam.co.jp/ab270048 for Japan)

For the rapid qualitative detection of His-tagged proteins.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

His-Tag Protein Expression Check Kit (ab270048) is a quick competitive immunochromatography test that allows users to verify and monitor successful expression of His-tagged recombinant proteins before undertaking purification, as well as identifying fractions of most interest before performing other more labor intensive techniques such as western blot or ELISA.

The assay provides a qualitative yes/no answer, although a semi-quantitative measurement of the protein abundance in the sample can be determined using internal references and controls.

A His-tagged protein on the "Test line" (T line) and a ULFA-tagged protein on the "Control line" (C line) are immobilized on the nitrocellulose strips.

40 nm Gold conjugated anti-HisTag antibody and 40 nm Gold conjugated anti-ULFA tag conjugates are dried on the conjugate pad and will selectively bind to the T and C line, respectively. The C line is assay-independent and should always appear as a strong red line; if it is not visible, the test is not valid and should be repeated.

His-Tag Protein Expression Check Kit (ab270048) is formulated as a competitive assay so that when the sample does not contain any His-tagged protein, the T line will appear as a strong red line (see Figure 1-A). On the contrary, the T line will not be visible (or appear as a pale red line) if the sample contains the His- tagged protein that competes with the binding of the Gold conjugated anti-HisTag antibody conjugate to the antigen immobilized on the T line (see Figure 1-B).

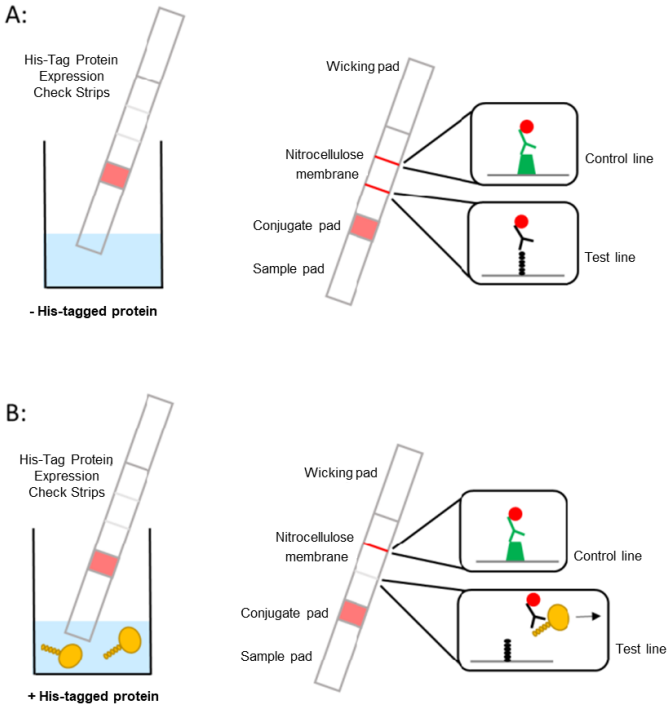

Figure 1. His-Tag Protein Expression Check Kit (ab270048) assay procedure. A: results in the absence of His-tagged protein, B: results in presence of His-tagged proteins.

2. Materials Supplied and Storage

Upon receipt, store kits at +4°C. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	30 tests	Storage temperature
10X Running Buffer	1 vials	+4°C
His-Tag Protein Expression Check Strips	30 Strips	+4°C

Not supplied: 96-wells low binding plate.

3. Technical Considerations

3.1 Sample Considerations:

The His-Tag Protein Expression Check Kit (ab270048) has been optimized with proteins containing accessible consecutive histidine regions at the amino- or carboxyl- terminus exposed in their native/ assay conformation.

The strips are compatible with cell culture media and lysate, as well as most common components used in purification processes (see table below).

Assay compatibility and interfering substances:

Substance	Compatability
1X PBS	Fully compatible
1X TBS	Fully compatible
RIPA buffer	Fully compatible
Tween20	< 5%
CHAPS	< 1%
Triton	< 5%
SDS	< 1%
Imidazole	< 125 mM
Guanidine HCl	< 100 mM
Urea	< 125 mM
DTT	< 100 mM
2-mercaptoethanol	< 100 mM
EDTA	< 100 mM

It is advisable to run the samples in duplicate.

A sample without the His-tagged protein should always be added as negative control: the strip will show the highest signal intensity on the T line that can be expected. The C line should always have the same signal intensity. To confirm the presence of expressed His-

tagged protein in the sample, a decrease in signal intensity of the T line should be seen, as compared to the negative control sample. Reference samples of known His-tagged protein concentration can be used for a semi-quantitative analysis.

3.2 Assay Considerations:

- The strips are single-use.
- Always store the unused strips in the closed desiccant pot to prevent moisture from compromising their functionality.
- Make sure the flow is consistent and that both the sample pad overlapping the conjugate pad, and the conjugate pad overlapping the nitrocellulose membrane are making physical contact. If not, a slight bend of the strip (avoiding touching or damaging the nitrocellulose membrane) is enough to restore the contact and establish a steady and effective flow.

4. Assay Procedure

4.1 Bring all the kit components to room temperature.

4.2 Dilute the 10X Running Buffer with distilled water down to 1X.

4.3 Only 80 µL of diluted sample are required for each strip. Dilute the lysate/sample containing the His-tagged protein down to 0.01-0.5 mg/mL in 1X Running Buffer; the appropriate range is protein dependent and should be determined empirically. If the protein concentration in the sample is unknown, perform serial 1:2 or 1:3 dilutions in 1X Running Buffer.

4.4 Load 80 µL of diluted protein into a 96-well clear plate (low protein binding) and dip the end of the strip with the sample pad into the liquid.

4.5 Wait 10-15 minutes for the T and C lines to develop (do not let the strips to dry out before checking the result).

4.6 Check the strips; the intensity of the T lines inversely correlates with the amount of His tagged protein present in the sample.

Technical Support

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